# METHOD FOR DIAGNOSING GENE


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Abstract of **JP2003245087**

<P>PROBLEM TO BE SOLVED: To provide a examination method for determining a risk of onset of two type diabetes mellitus and to provide a probe used in the method or a nucleic acid useful as a prove or a primer which can be used for the method. <P>SOLUTION: A genetic polymorphism at a position of from the 1038th to 1048th of a second intron of 3 gene of angiopoietin in a human genome DNA is specifically detected by detecting a sample containing the human genome DNA and using a genetic polymorphism analyzing method. <P>COPYRIGHT: (C)2003,JPO

Notes:
1. Untranslatable words are replaced with asterisks (****).
2. Texts in the figures are not translated and shown as it is.

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## CLAIMS

[Claim(s)]

[Claim 1] Process which extracts Genome DNA from method:1 sample which is the way the subject who inspected the sample containing a human genome DNA and offered this sample evaluates the danger which shows the symptoms of 2 type diabetes, and is characterized by including the following processes 1 and 2;

2) [ the nucleotide sequence in the 1038th to the 1049th position (nucleotide number 1038-1049 of the arrangement number 1 of an arrangement table) of the 2nd intron of ANJIOPOECHIN related protein 3 gene in the genome DNA obtained by the above 1 ] The detection process of gene many models of judging whether it being in agreement with the arrangement number 1 of an arrangement table, or the position concerned being replaced by the nucleotide sequence shown in the arrangement number 2 of an arrangement table.

[Claim 2] The detection process of gene many models The RFLP method, the PCR-SSCP method, an ASO hybridization, The direct sequence method, the ARMS (Amplification Refracting Mutation System) method, A denaturing agent concentration gradient gel electrophoresis method, a RNaseA method of section, a chemistry method of section, The DOL (Dye-labeled Oligonucleotide Ligation) method, TaqMan The PCR method, the invader method, MALDI-TOF/MS (Matrix) Assisted Laser Desorption-time [ that it is a process by one or two methods or more which are chosen from the group which consists of the of Flight/Mass Spectrometry method and the TDI (Template-directed Dye-terminator Incorporation) method ] The method according to claim 1 by which it is characterized.

[Claim 3] The method according to claim 1 or 2 that the detection process of gene many models is characterized by being the direct sequence method.

[Claim 4] The nucleic acid which consists of a nucleotide sequence chosen from the group which consists of following **-**, or nucleotide sequence shown in the nucleotide numbers 130-2462 of the arrangement number 1 of the fluorescence pigment sign thing:** arrangement

table;
** 15 nucleotides or the continuous partial nucleotide sequence beyond it in a nucleotide sequence given in the above-mentioned **;
** [ the end of the nucleotide sequence of any one description of the anti sense arrangement / of the nucleotide sequence of any one description of the above-mentioned ** or the ** /;, and ** above-mentioned **, or the ** ] The nucleotide sequence to which arbitrary linker arrangement was added (however, the above-mentioned **-** shall read "t" under the arrangement shown in the arrangement number 1 of an arrangement table, or its anti sense arrangement as "u", when nucleic acid is RNA).
[Claim 5] Nucleic acid according to claim 4 characterized by being DNA.
[Claim 6] DNA which consists of a nucleotide sequence shown in the arrangement number 5 of an arrangement table.

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to the inspection method of the gene many models for judging the danger which shows the symptoms of 2 type diabetes.
[0002]
[Description of the Prior Art] The patient load is increasing in recent years, and diabetes attracts attention as one of the geriatric diseases. 2 type diabetes (noninsulin dependent diabetes mellitus, NIDDM) is the type of many diabetes to Japanese people, and early detection and early treatment are important for it also from a point of the prognosis.
[0003] However, the origin of 2 type diabetes is various, and the knowledge about the cause of it being expected and getting is scarce. As a cause in 2 type diabetes that an insulin operation is insufficient, the abnormalities of an insulin susceptibility mechanism and the fall of insulin secretion are cited. Although the former, i.e., insulin resistance, is the main causes of 2 type diabetes, there are not little many in Europe and America in Japan, also when considering insulin secretion insufficiency as the main causes.
[0004] On the other hand, the effect of a medicine of the medicine or prediction of side effects is being attained by gene diagnosis by investigating the effect of a medicine of gene many models and a medicine, or the relation of side effects by progress of the latest Pharma KOJIENO mix research. Moreover, prior diagnosis of some diseases and a prognostic judgment are being attained by investigating the relation between gene many models and a disease. As a former example, the example of the gene many models of drug metabolism enzyme is given. Activity is known for SHITOKUROMU P4501A2, SHITOKUROMU P4502A6, SHITOKUROMU P4502C9, SHITOKUROMU P4502C19, SHITOKUROMU P4502D6,

SHITOKUROMU P4502E1, etc. as an increase or many models of the drug metabolism enzyme which decreases in number. moreover, a group called **** enzyme, such as CHIOPURIN methyl transferase, N-ASECHIRU Torrance FERAZE, UDP-GURUKU uronosyl Torrance FERAZE, and GURUTA thione S-Torrance FERAZE, -- gene many models exist also in an enzyme group, and there are many models It is reported that activity decreases (for example, refer to nonpatent literature 1.). Moreover, many found-out disease cause genes are reported by multi-model analysis research as a latter example. As HLA as a cause gene of for example, ** chronic ulcerative colitis, and the cause gene of ** rheumatoid arthritis Dopamine D3 receptor as APOE4 as a cause gene of **TCRalpha and ** ARUTSUAIMA disease, and a cause gene of ** schizophrenia, ** The leptin as the blood coagulation factor VII as Try H as a cause gene of manic-depressive insanity, the angiotensin precursor as a cause gene of ** albumin ****, and a cause gene of ** myocardial infarction and a cause gene of ** overweight etc. is mentioned (for example, refer to nonpatent literature 2.).

[0005] Moreover, although it was thought that TNF-alpha was an important substance which participates in symptoms formation of various inflammatory diseases, the many models which rise TNF-alpha gene expression in the 5' end side upper stream region of a TNF-alpha gene were found out recently (for example, refer to nonpatent literature 3.). Since these many models are what accelerates the amount of TNF-alpha gene expression, prior diagnosis of the disease in which TNF-alpha, such as juvenile rheumatoid arthritis, rheumatoid arthritis, and diabetes, participates by gene diagnosis is possible for them (for example, refer to patent documents 1.).

[0006] The relation of the variation of a gene and diabetes development of symptoms is considered also about diabetes. As the gene which is clear by the present and which is supposed that the symptoms of diabetes are shown by independent abnormalities, There are an insulin, an insulin receptor, an insulin promotor factor, guru KOKINAZE, a HEPATOSAITONYU Clare factor, AMIRIN, skeletal muscle glycogen synthase, abnormalities in a mitochondrial gene, etc. Moreover, it is a gene considered to participate in development of symptoms of NIDDM. Insulin receptor subSUTOREITO, a glucose transporter, Mitochondria glycerophosphate dehydrogenase, fatty acid binding protein, an adrenalin receptor, a glucagon receptor, a potassium channel, CD38, etc. are mentioned (for example, refer to nonpatent literature 4.).

[0007] Moreover, it was reported that the Arg140Trp many models in CD38 raise a diabetic risk these days (for example, refer to nonpatent literature 5.). It is thought that they are useful to diabetic risk diagnosis of receiving since these many models are found out by the Japanese diabetic by about 13% of frequency.

[0008] However, about the relation between the many models of ANJIOPOECHIN related protein 3 gene, and 2 type diabetes, it is not known at all. Moreover, it is supposed that

symptoms develop 2 type diabetes by external factors, such as two or more gene many models or eating habits, rather than producing with the variation and the many models of a single gene. The development-of-symptoms mechanism has many points [ **** / un-], and research on the gene which will participate in diabetic development of symptoms further from now on, or its many models is desired.

[0009]

[Patent documents 1] Re-table No. 054361 [ 98 to ] gazette [Nonpatent literature 1] The volume for Yusuke Nakamura, "the strategy of SNP gene many models", Nakayama Shoten, 2000 [Nonpatent literature 2] "Nature genetics (Nature Genetics)", 1999, the 22nd volume, p.139-144 [Nonpatent literature 3] "Tissue anti GENZU (Tissue Antigens)", 1998, the 51st volume, p.605-612 [Nonpatent literature 4] The Kadowaki ****, "the diabetic front line", 1997, Yodosha [Nonpatent literature 5] "Diabetologia (Diabetologia)", 1998, the 41st volume, p.1024-1028 [0010]

[Problem(s) to be Solved by the Invention] The purpose of this invention is to offer the probe which may be used for the inspection method and this method for judging the danger of development of symptoms of 2 type diabetes, or nucleic acid useful as a primer.

[0011]

[Means for Solving the Problem] This invention (1) Process which extracts Genome DNA from method:1 sample which is the way the subject who inspected the sample containing a human genome DNA and offered this sample evaluates the danger which shows the symptoms of 2 type diabetes, and is characterized by including the following processes 1 and 2;

2) [ the nucleotide sequence in the 1038th to the 1049th position (nucleotide number 1038-1049 of the arrangement number 1 of an arrangement table) of the 2nd intron of ANJIOPOECHIN related protein 3 gene in the genome DNA obtained by the above 1 ] Judge whether it is in agreement with the arrangement number 1 of an arrangement table, or the position concerned is replaced by the nucleotide sequence shown in the arrangement number 2 of an arrangement table. Detection process (2) of gene many models The detection process of gene many models The RFLP method, The PCR-SSCP method, an ASO hybridization, the direct sequence method, The ARMS (Amplification Refracting Mutation System) method, A denaturing agent concentration gradient gel electrophoresis method, a RNaseA method of section, a chemistry method of section, the DOL (Dye-labeled Oligonucleotide Ligation) method, TaqMan The PCR method, the invader method, MALDI-TOF/MS (Matrix) Assisted Laser Desorption-time [ that it is a process by one or two methods or more which are chosen from the group which consists of the of Flight/Mass Spectrometry method and the TDI (Template-directed Dye-terminator Incorporation) method ] Method (3) given in (1) by which it is characterized [ the detection process of gene many models ] (1) characterized by being the direct sequence method, or a method given in (2), (4) The nucleic acid which consists of a

nucleotide sequence chosen from the group which consists of following **-**, or nucleotide sequence shown in the nucleotide numbers 130-2462 of the arrangement number 1 of the fluorescence pigment sign thing:** arrangement table;
** 15 nucleotides or the continuous partial nucleotide sequence beyond it in a nucleotide sequence given in the above-mentioned **;
** [ the end of the nucleotide sequence of any one description of the anti sense arrangement / of the nucleotide sequence of any one description of the above-mentioned ** or the ** /;, and ** above-mentioned **, or the ** ] The nucleotide sequence to which arbitrary linker arrangement was added (however, [ the above-mentioned **-** ]) When nucleic acid is RNA, shall read "t" under the arrangement shown in the arrangement number 1 of an arrangement table, or its anti sense arrangement as "u." (5) Nucleic acid (6) given in (4) characterized by being DNA It is related with DNA which consists of a nucleotide sequence shown in the arrangement number 5 of an arrangement table.
[0012] The result of having investigated the frequency of the gene many models which exist in the ANJIOPOECHIN related protein 3 gene in the DNA sample which this invention persons are the purposes which search for a diabetic disease related human gene, and was extracted from the diabetic and the healthy person, By solving that the gene many models which appear by low frequency exist in a diabetic, and inspecting the gene many models, the subject found out that the potential danger which shows the symptoms of diabetes could be judged by high probability, and completed this invention.
[0013] In this invention, it is used as a term containing both many models covering the arrangement which serves as "gene many models" from what is called single nucleotide many models (single nucleotide polymorphism: single nucleotide polymorphism:SNP) and two or more nucleotides. When [ namely, ] based on the genome sequence of a certain one individual in the group concerned in a human group To the specific part in the genome in other 1 or two or more individuals, 1 or the substitution of two or more nucleotides, [ that it is not what is depended on the mutation which variation, such as deficiency, insertion, transposition, and reverse grade, existed, and the variation produced in 1 or two or more individuals concerned / appear / it / by the frequency of the grade statistically judged to be certain ] Or if it is proved in family line that it is not the mutation in the individual concerned, the variation will be included in the "gene many models" in this invention, and it will deal in it.
[0014]
[Embodiment of the Invention] Prepare the method of this invention and the genome DNA sample corresponding to the gene multi-model analysis method later mentioned from the material containing a human genome DNA subsequently Using this gene multi-model analysis method, the inside of the genome DNA concerned, It can carry out by detecting specifically the gene many models in the 1038th to the 1049th position (nucleotide number 1038-1049 of the

arrangement number 1 of an arrangement table) of the 2nd intron of ANJIOPOECHIN related protein 3 gene.

[0015] Although all the cells (except for a productive cell) extracted from the subject, tissue, internal organs, etc. can be used as a material for obtaining Genome DNA, it is the white corpuscles or monocyte preferably separated from peripheral blood, and they are white corpuscles most suitably. Such material may be extracted by the method usually used in a clinical examination.

[0016] For example, when using white corpuscles, white corpuscles are separated from the peripheral blood first extracted from the subject by the well-known method. Subsequently, after adding pro TEINAZE K and dodecyl sodium sulfate (SDS) to the obtained white corpuscles, decomposing and denaturing protein, Genome DNA (RNA is included) is obtained by performing phenol/chloroform extraction. RNA is removable with RNase if needed. However, in extraction of the genome DNA from the sample which this invention is not limited to this but contains a human genome DNA In the technical field of this invention, it is the well-known method (for example), i.e., literature. Sambrook and J.et al. (1989): "Molecular Cloning The method indicated to :A Laboratory Manual"(2nd Ed.) Cold Spring Harbor Laboratory and NY, The method of using a commercial DNA extraction kit etc. can also be used preferably.

[0017] Subsequently, although the diabetes and the gene many models with very deep relation which were solved by this invention persons are detected about the obtained genome DNA, the following methods can be mentioned as the typical detection method.

[0018] 1) When the RFLP (restriction enzyme cutting fragment length many models) method gene polymorphic area is contained in a restriction enzyme recognition site, the gene many types concerned of judgment is possible by the difference in the length of the DNA fragment produced by being digested by the restriction enzyme. In this case, the method of analyzing the size of the DNA fragment which cut the method of performing a SAZAMBU lot for the ** genome DNA after decomposition by a restriction enzyme, and the DNA fragment containing ** polymorphic area by the restriction enzyme after PCR amplification, and was cut by electrophoresis is mentioned. ** as a probe used in a method The DNA fragment (that by which the sign was carried out with an isotope, biotin, or a fluorescence pigment) which is equivalent to the arrangement over which 5 'end side and 3' end side goes by each about 0.05 to 2 kb from the polymorphic area including the target polymorphic area is desirable. Moreover, the PCR primer used for the method of ** is the oligonucleotide of 15-30mer for amplifying preferably the DNA fragment of about 0.05 to 4 kb containing a polymorphic area.

[0019] 2) The PCR-SSCP method (single-strand-conformation-polymorphism analysis) By PCR, after amplification, the PCR-SSCP method carries out heat denaturation of the DNA fragment containing a gene polymorphic area, and [ with electrophoresis ] It is the method of separating the 1 chain DNA with which high order structures differ [bio-technics

(Biotechniques), 16, 296-297 (1994), bio-TEKUNIKUSU (Biotechniques), 21, and 510-514 (1996)]. Typing of many models is possible by analyzing the pattern by the existence of gene many models, since the migration distances of the 1 chain DNA differ. The primer for PCR amplification is the oligonucleotide of 15-30mer for amplifying the DNA fragment of about 50 to 500 bp which contains preferably the polymorphic area which carried out the sign of the 5' end with the fluorescence pigment.

[0020] 3) [ the ASO (Allele Specific Oligonucleotide) hybridization method ASO hybridization method ] The DOTTOBU lot of the PCR product containing a gene polymorphic area is carried out to base materials, such as a nylon filter. it has a nucleotide sequence corresponding to each gene many models -- usually -- the synthetic oligonucleotide probe (a signal -- obtaining -- radioisotope --) of about 15-25 mer [ with the washing operation which applied to Tm value of the probe correspondingly after the hybridization / that the sign by biotin or a fluorescence pigment is required ] It is the method of detecting the mismatch of one nucleotide (a hybrid separating if there is a mismatch) [a KURINIKA KIMIKA actor (Clin.Chim.Acta), 189, and 153-157 (1990)]. The PCR primer used by this method is the oligonucleotide of 15-30mer for amplifying preferably the DNA fragment of about 0.05 to 4 kb containing a polymorphic area.

[0021] 4) [ the direct sequence method direct sequence method ] It is the method of analyzing the nucleotide sequence of DNA amplified by the PCR method after amplification in the DNA fragment containing a gene polymorphic area by the direct DAIDEOKISHI method [bio-technics (Biotechniques), 11, and 246-249 (1991)]. The PCR primer used by this method is the oligonucleotide of 15-30mer for amplifying preferably the DNA fragment of about 0.05 to 4 kb containing a polymorphic area. Moreover, as a sequence primer, the oligonucleotide of 15-30mer which is equivalent to the position by the side of 5' end about 50-300 nucleotides from a polymorphic area is used preferably.

[0022] 5) In ARMS (Amplification Refracting Mutation System) method PCR, after ANIRU [ Mold DNA / a primer ], a complementary strand DNA is compounded by DNA poly MERAZE at the 5'3 from end side' end side. If a mismatch is in 3' end nucleotide of a primer, the efficiency of PCR will fall and it will become undetectable in electrophoresis. The ARMS method performs PCR using a primer which becomes complementary at the variation nucleotide which 3' end nucleotide tends to detect. [NUKUREIKKU ASHIZZU research which detects gene many models by judging the existence of an amplification product and which is a method (Nuc.Acids.Res.), 19 3561-3567 (1991), NUKUREIKKU ASHIZZU research (Nuc.Acids.Res.) 20, 4831-4837 (1992)]. [ the primer used for PCR in the ARMS method ] It is the oligonucleotide of 15-30mer of the portion in which one was designed so that a polymorphic area might be located in 3' end and which are the oligonucleotide of 15-30mer preferably, and another is desirable, and separated about 0.05-2 kb from the polymorphic area.

[0023] 6) [ the denaturing agent concentration gradient gel electrophoresis (Denaturing Gradient Gel Electrophoresis and the following call it "DGGE") method DGGE method ] HETERODEYUPUREKKUSU which has a mismatch in a PCR product is the way gay duplex **** also detects gene many models using **** being easy [bio-technics (Biotechniqus), 27, and 1016-1018 (1999)]. Since the mobility in gel electrophoresis falls as hetero duplex ** and **** progress If the density gradient of urea and HORUMU amide is set up into the polyacrylamide gel to be used, the difference of the mobility of gay duplex ** will be emphasized further, and existence of the double stranded DNA containing a mismatch, i.e., existence of variation, will be detected. The PCR primer used by this method is the oligonucleotide of 15-30mer for usually amplifying the DNA fragment of about 0.05 to 0.5 kb containing a polymorphic area.
[0024] 7) The RNaseA method of section RNaseA (RNA dialytic ferment) has the characteristic which does not decompose RNA or the RNA/DNA complex of 2 chains, but decomposes only RNA of 1 chain. Therefore, if it is made to hybridize with the PCR product which denatured and used as 1 chain the RNA probe which carried out the sign of the DNA fragment containing a polymorphic area by the isotope after amplification with PCR and electrophoresis separates after RNaseA processing Since a variant and the hybridized RNA probe are cut by a mismatch part, it is detectable as two bands [DNA and cell biology (DNA Cell.Biol.), 14, and 87-94 (1995)]. As a RNA probe used by this method, the thing of usual 15-30mer containing a polymorphic area is desirable.
[0025] 8) Sugar will be cut, if the DNA fragment containing a chemistry method-of-section polymorphic area is separately embellished with osmium tetra-OKISHIDO to hydronalium KISHIRU amine and "t (thymine)" after amplification to "c (cytosine)" of the mismatch part of the double stranded DNA by PCR and PIPERIJIN processing is carried out. Electrophoresis is carried out, and if the size of the probe is short, it will mean that variation was detected after making 2 chains form using a sign probe and performing the above-mentioned processing [bio-technics (Biotechniques), 21, and 216-218 (1996)]. The PCR primer used by this method is the oligonucleotide of 15-30mer for usually amplifying the DNA fragment of about 0.05 to 4 kb containing a polymorphic area.
[0026] 9) [ the DOL (Dye-labeled Oligonucleotide Ligation) method DOL method ] DAIPURAIMA included to the base in front of the polymorphic area by which the fluorescence sign was carried out after making the DNA fragment containing gene many models amplify by PCR, It is the method with which Di Terminator by which the sign was carried out to each allele with the specific fluorescence pigment is made to connect with heat-resistant DNA ligase [a genome research (Genome Res.), 8, and 549-556 (1998)]. The PCR primer used at this time is the oligonucleotide of 15-30mer for amplifying the DNA fragment of about 0.05 to 2 kb which usually contains a polymorphic area.
[0027] 10) TaqMan PCR method TaqMan [ the PCR method ] [JIENETIKU analysis which is a

method using PCR by the allele specific oligonucleotide (TaqMan probe) and TaqDNA poly MERAZE which carried out the fluorescence sign (Genet.Anal.), 14 143-149 (1999), Journal OBU clinical microbiology (J.Clin.Microbiol.), 34, 2933-2936 (1996)]. The PCR primer used by this method is the oligonucleotide of 15-30mer for amplifying the DNA fragment of about 0.05 to 2 kb which usually contains a polymorphic area. Moreover, a TaqMan probe is a length of about 15-30 bases containing a polymorphic area, the sign of the 5' end is carried out with the fluorescence reporter pigment, and the sign of the 3' end is simultaneously carried out by the quencher (optical quenching substance). By using this probe, detection of nucleotide change of a wild type and a variant is possible.

[0028] 11) The invader method invader method is TaqMan. The [science (Science) which is the method of typing by carrying out the hybridization of an allele specific oligonucleotide and the mold like the PCR method, 5109 778-783 (1993), Journal of Biological Chemistry (J.Biol.Chem.), 30 21387-21394 (1999), The Nature biotechnology (Nat.Biotechnol.), 17,292-296 (1999)].

[0029] 12) [ the MALDI-TOF/MS method (Matrix Assisted Laser Desorption-time of Flight/Mass Spectrometry) method MALDI-TOF/MS method ] Compound 1 chain oligonucleotide containing a different nucleotide corresponding to the allele of gene many models, and the mass is measured. [genome research which is the method of typing by detecting the difference with mass spectrograph (Genome Res.), 7 378-388 (1997), Euro PIAN journal OBU Clinical Chemistry and clinical biochemistry (Eur.J.Clin.Chem.Clin.Biochem.) 35, 545-548 (1997)]. By the MALDI-TOF/MS method, the DNA fragment which contains a polymorphic area first is amplified by the PCR method, and a specific DNA extension reaction thing is analyzed with a mass spectrum to each allele after that by the extension reaction using the primer which adjoins a polymorphic area. It is used at this time and a PCR primer is the oligonucleotide of 15-30mer for amplifying the DNA fragment of about 0.05 to 0.5 kb which usually contains a polymorphic area. Moreover, the oligonucleotide of 30mer(s) is used for the primer for detecting many models from 15 which adjoined the polymorphic area.

[0030] 13) [ the TDI (Template-directed Dye-terminator Incorporation) method TDI method ] After making the DNA fragment containing gene many models amplify by PCR, using the primer designed just before the polymorphic area [ with a primer extension reaction ] [proceedings OBU, the THE National Academy of Sciences OBU THE YUNAITETTO States OBU United States which make the DAIDEOKISHI nucleotide by which the sign was carried out with a different fluorescence pigment corresponding to each allele take into a polymorphic area and which are a method (Proc.Natl.Acad.Sci.USA), 94, 10756-10761 (1997)]. A primer extension product is analyzed using a DNA sequencer (the ABI prism 377, applied bio-systems company make) etc. The PCR primer used at this time is the oligonucleotide of 15-30mer for amplifying the DNA fragment of about 0.05 to 1 kb which usually contains a polymorphic area.

[0031] Among these, although the direct sequence method is the most suitable detection method in the method of this invention, the method of this invention is not limited to the thing using the above-mentioned method, but other well-known gene multi-model detection methods may be used for the method of this invention. Moreover, in the method of this invention, these gene multi-model detection methods may be used combining two or more methods, even if used independently.

[0032] The probe or primer which has singularity is used for the part in which the gene many models of the purpose in a genome exist in any case, or the arrangement of the circumference of it in the detection method of the above gene many models. It is the nucleotide sequence shown in the nucleotide numbers 130-2462 of the arrangement number 1 of :** arrangement table where what suited each aforementioned detection method is chosen from the group which consists of **-** indicated below as arrangement of the nucleic acid which may be used as a primer or a probe in the method of this invention.;

** 15 nucleotides or the continuous partial nucleotide sequence beyond it in a nucleotide sequence given in the above-mentioned **;

** [ the end of the nucleotide sequence of any one description of the anti sense arrangement / of the nucleotide sequence of any one description of the above-mentioned ** or the ** /;, and ** above-mentioned **, or the ** ] The nucleotide sequence to which linker arrangement required for the above mentioned multi-model detection method was added (However, the above-mentioned **-** shall read "t" under the arrangement shown in the arrangement number 1 of an arrangement table, or its anti sense arrangement as "u", when a probe or a primer is oligo RIBONUKUREORIDO or a PORIRIBO nucleotide) .

[0033] Moreover, that by which signs, such as a fluorescence pigment for detection, were added to the end of the nucleotide sequence chosen from the group which consists of the above-mentioned **-** is also included by this invention.

[0034] [ arrangement / of the above-mentioned ** / the partial arrangement or its anti sense arrangement ] [ as long as it is usable in each gene multi-model detection method, may choose what kind of portion, but ] [ whether the 1049th (nucleotide number 1038-1049 of the arrangement number 1 of an arrangement table) is replaced from the 1038th of the 2nd intron of ANJIOPOECHIN related protein 3 gene by the nucleotide sequence shown in the arrangement number 2 of an arrangement table ] [ a thing suitable as a combination of the range of the arrangement chosen as the PCR primers for detecting ] as a :sense strand primer which is as indicating below The inside of the nucleotide sequence suitably shown in the nucleotide numbers 538-1037 of the arrangement number 1 of an arrangement table, The inside of the nucleotide sequence more suitably shown in these nucleotide numbers 838-1037, 15 nucleotides or the continuous nucleotide sequence beyond it (preferably 15 to 30 nucleotide); as an antisense strand primer It is in the nucleotide sequence more suitably

shown in these nucleotide numbers 1050-1249 among the nucleotide sequence suitably shown in the nucleotide numbers 1050-1549 of the arrangement number 1 of an arrangement table, 15 nucleotides or anti sense arrangement of the continuous nucleotide sequence beyond it (preferably 15 to 30 nucleotide).

[0035] moreover, as a primer used in the direct sequence method The inside of the nucleotide sequence shown in the nucleotide number 431-1037 of the arrangement number 1 of an arrangement table, 15 nucleotides or the continuous nucleotide sequence beyond it (preferably 15 to 30 nucleotide), Or the inside of the nucleotide sequence shown in these nucleotide numbers 1050-1650, The oligonucleotide which consists of anti sense arrangement of 15 nucleotides or the continuous nucleotide sequence beyond it (preferably 15 to 30 nucleotide) is suitable, and the oligonucleotide which consists of a nucleotide sequence shown in the arrangement number 5 of an arrangement table is the most suitable.

[0036] The nucleic acid which may be used as the above primers or a probe can be obtained by carrying out chemical synthesis using the well-known method in the technical field of this invention.

[0037] It has become clear from the statistics analysis result of the gene multi-model detection result of the diabetic and healthy person by this invention persons for the judgment of the potential danger which shows the symptoms of diabetes in high probability to be possible for the method of this invention. Namely, the result of having investigated gene many models by the method of this invention using the sample extracted from a certain subject, The 1038th to the 1049th nucleotide sequence (nucleotide number 1038-1049 of the arrangement number 1 of an arrangement table) of the 2nd intron of ANJIOPOECHIN related protein 3 gene is following nucleotide sequence:5'-ttttctaaacaag-3' (arrangement number 2 of an arrangement table).

To the subject who has the gene come out of and replaced by hetero, information that the danger which shows the symptoms of 2 type diabetes is relatively low can be offered.

[0038] Since the measure for notifying of that and preventing diabetes beforehand by this invention to the subject it was proved that he is that the danger which shows the symptoms of diabetes from the above-mentioned judging standard is relatively high can be taken, this invention is very useful as an inspection method for preventing this illness.

[0039]

[Example] Although an example is indicated below and this invention is explained to it still more concretely, this invention is not limited to these.

[0040] Example 1 Each subject's genome DNA sample was extracted by making into start material the white corpuscles separated from peripheral blood of the manufacture healthy persons of the genome DNA from peripheral blood, and 2 type diabetic, and using an automatic DNA extracting apparatus (NA-1000, Kurabo Industries, Ltd. make). 260nm ****** of

each obtained sample was measured, and the DNA concentration of each sample was computed. Then, Genome DNA was diluted to 5 ng/mu l using sterilization distilled water, and it was considered as the sample of PCR.

[0041] Example 2 The DNA fragment containing the polymorphic area on ANJIOPOECHIN related protein 3 gene was amplified by the method of indicating below by using as a mold each subject's genome DNA sample obtained in the amplification example 1 of the DNA fragment containing the many models by the PCR method.

[0042] A PCR primer is primer No.1:5'-gcaggctctgaagctaataaactac-3' (arrangement number 3 of an arrangement table) which consists of the following arrangement, And primer No.2:5'-ctagaacaagtcctaagaataggga-3' (arrangement number 4 of an arrangement table) which consists of the following arrangement was used. In addition, primer No.1 is the arrangement number 1 of an arrangement table. ([ ANJIOPOECHIN related protein 3 gene / the 2nd intron ] the 3rd exon) It is equivalent to the nucleotide sequence shown in the nucleotide number 130-154 under arrangement which consists of the 3rd intron, and primer No.2 are equivalent to the anti sense arrangement of the nucleotide sequence shown in the nucleotide number 2438-2462 in the arrangement number 1 of an arrangement table.

[0043] The PCR method was performed on condition of the following. 2.5mM(s) of 4microl dNTP, 25mM(s) of 4microl Magnesium sulfate, 3.2microM of 6.25microl 3.2microM of primer No.1 or 6.25microl The 5 ng/mu l genome DNA of primer No.2 or 8microl The 10xPCR reaction buffer solution (it attaches to TaKaRa LA Taq (made by TAKARA SHUZO [ CO., LTD. ] CO., LTD.)) of 4microl, sterile water of 7.1microl, and Taq poly MERAZE of 0.4microl (Takara LA Taq (made by TAKARA SHUZO [ CO., LTD. ] CO., LTD.), five units /mul) By the system of reaction of mixed a total of 40microl, at 94 degrees C, it carried out for 45 seconds at 60 degrees C for 45 seconds, 72 degrees C performed the cycle of 1.5 minutes 30 times, and the DNA fragment of about 2.3 kbp(s) containing a polymorphic area was amplified.

[0044] Example 3 Determination of the nucleotide sequence for identifying the polymorphic area of detection ANJIOPOECHIN related protein 3 gene of the many models of ANJIOPOECHIN related protein 3 gene, Use as a mold the fragment obtained in the example 2, and following nucleotide sequence:5'-ggattactag aaatagtgtg cctca-3' (arrangement number 5 of an arrangement table) is used as a primer. The DNA sequencer (ABI PRISM 3700, applied bio-systems company) was used for the passage, and it usually went it for it.

[0045] From the 1038th to the 1049th [ as a result, ] of the 2nd intron of ANJIOPOECHIN related protein 3 gene The genotype (whose nucleotide number 1038-1049 of the arrangement number 1 of an arrangement table) corresponds with the arrangement number 1 of an arrangement table (henceforth "the many models 1"), It was checked that the many models with which the part concerned consists of genotype (henceforth "the many models 2") replaced by the nucleotide sequence shown in the arrangement number 2 of an arrangement table

exist.

[0046] Example 4 The relation of the many models and diabetes which were identified in the related example 3 of many models and diabetes was analyzed by the connection analyzing method (the association method).

[0047] Number of men and women and the average age of a healthy person (101 persons) and 2 type diabetic (140 persons) which were made applicable to analysis are shown in Table 1.

[0048]

[Table 1]

| | 被験者数 | | | 平均年齢±S.D. |
|---|---|---|---|---|
| | 男性 | 女性 | 全体 | |
| 健常人 | 54 | 47 | 101 | 69.7±10.4 |
| 2型糖尿病 | 86 | 54 | 140 | 71.1±11.0 |

When statistical analysis was conducted about distribution of the man-and-woman ratio in both groups, and age, and the average age, the significant difference was not seen in any. In addition, as the statistical analysis technique, Bartlett official approval was used about the difference in the distribution of chi (paddle) square official approval of age about the difference in a man-and-woman ratio, and t official approval was used about the difference in the average age. The frequency of the many models identified in the example 3 was computed by having conducted multi-model analysis by the method indicated in the examples 1-3 about both groups, and relation with diabetes was analyzed. In addition, as the statistical analysis technique, official approval was used chi (paddle) square. A result is shown in Table 2.

[0049]

[Table 2]

| | 被験者数 | 遺伝子型 | | | アレル頻度 | | P値 ($\chi^2$検定) | 有意差 |
|---|---|---|---|---|---|---|---|---|
| | | 多型1/多型1 | 多型1/多型2 | 多型2/多型2 | 多型1 | 多型2 | | |
| 健常人 | 101 | 97 | 4 | 0 | 198 | 4 | - | - |
| 2型糖尿病 | 140 | 140 | 0 | 0 | 280 | 0 | 0.0181 | あり |

From the result of Table 2, it was shown in the healthy person and 2 type diabetic that the frequency of each genotype in the many models identified in the example 3 differs intentionally statistically. This shows that the relative danger which shows the symptoms of 2 type diabetes can be judged by investigating the gene many models concerned.

[0050]

[Effect of the Invention] Like the above, the probe which may be used for the initial inspection method for judging the danger of development of symptoms of 2 type diabetes by this

invention and this method, or nucleic acid useful as a primer was offered. This invention is useful as a material used for the inspection method and this method for diabetic prevention.
[0051]
[Layout Table]
SEQUENCE LISTING<110> Sankyo Company and Limited <120> Method for Genetic Diagnosis <130> 2002143SU<150> JP 2001-356969<151> 2001-11-22 <150> JP 2001-384102<151> 2001-12-18 <160> 5<170> PatentIn Ver.2.1<210> 1 <211> 2962<212> DNA <213> Homo sapiens <223> Inventor: Yasumo, Hiroaki; Koishi, Ryuta; Furukawa and Hidehiko <400> 1 gtaagtcagt attttaatgg tatgtcccat ctttcacaca ggtctgtaaa aacactgaat 60 cctaaaatta tttacaagct ttaactggat catgagtaaa attatcacat cagcataact 120 gttaaaattg caggc tctga agctaataaa ctacctgcat ttaaaccatg gctctaaaac 180 Tttgtgtgac Cttgaataaa ttacttcacc cctttatctc tcagtttcct cacatatact 240 Acaaagataa Taacagaact tataggatta ttgtaagaaa aaaaattaat tcatagcagc 300 Caatgtcatc Ttactaaaat tcaaattaga tcatgtttct ctttgctcaa aaccacacaa 360 Tagctttcca Tttcactcat attggctctt tagaccaaga ttacccaacc cttcgtcatc 420 Tcactgactt Cacctcctct actctagtta ttctgaccgc tttaccagta ttcaaacaca 480 tcaaacatac tgccacctca aagcctttgc ccttgttgtt tcctctaact ggaacgctct 540 tctgccctgg tatctacgtg gcccactctc tgatttccct tagggtcgtt atcaaacaaa 600 aaattcccaa tgaagactta caaggtcact taaccaaaaa tcacaaccgc ctggtcccat 660 ccctgaaaac ttctacttcc ttagctactt ttctcctgca cactcacctt tatttaacat 720 aacataaatt ttagttattt atctcttcta ttcctgcact aaaatgtaag ctctgtgaat 780 acagggattt tttccattat cttcatattt tccattattt gtatatactc cagaatatag 840 aatactgtat ggcacacagt aggcatttct gttgaattaa taaatgtaat gtcatattca 900 cacagaagcg tgtgctatga ttattattac ttggattact agaaatagtg tgcctcataa 960 ttaaag gtca acattcaaca atgtaattaa tctacaatgt aaacatctgg tgaagtgaca 1020 gagggaagca cttgtttaga aaaaagctat gtcagaatcc atgtattcta atatgcagta 1080 caatagttta aaaatattaa taatactctc aaacagctat tcaagaggat tcaaaaaaca 1140 taatataaac tcagagaaac tggtaaacaa aatcattttc aagagatata aaacaaatat 1200 tattaccaat ttccactaaa caaacataat gttagtagtg ctgctaaaag gttttttatc 1260 aactactttt ggtttccata ctttccttct tatgatgtta ttattctaaa ttcttttcaa 1320 ttatatcttt tactatgatt aaatgaacct gctccccaaa gcaaaatgtt actatagtaa 1380 t atacattgt gtctaaaaat aaaaatgtgt gaagaaacca aaacaatgaa tttctgagtt 1440 Ggaagaagag Ttagatcatt taacttttctc atatttaaat taaaaaaaca aaactctaaa 1500 Aatttaagta Actttaagat cacatagtta cttagtagaa aagagtaata cccagcaagc 1560 Aaactttaca Atagatcctt ttaaataagg tcctaggaaa tatcattcat gccagcatca 1620 Aaaaactaac Actaataatg caagatatta tatattctgc ttttcttact gtcaatgaga 1680 Aaaactatca Ttcaataaat tgcaaaccca acacacttaa ataaaaataa aatgttactg 1740 ctaaactaac gataaactac tgaatatata gaaagtaagc " ** aacaaactt gccaacctgc 1 800 caacatctac agatatgttt acaggtcaaa aattatcaaa ttatcaagaa agcctggttc 1860 Aaattatgta Ttatgtcttt atcacaggtc tgaagatcag taagacctaa aactgaaaat 1920 Tattaaactt Aaaatctgaa cagaatatca aatatatttt attcatataa ataaaagaat 1980 Acattacaat Attctaagca aagcagtctc tacttttggc cttgctctgt tttccgacca 2040 Atgtctgctt Ttttgccttg ctttatttttt ttatcttatt aaataatgtc cctgattaaa 2100 Tattttgaga Acaggtaatc tgtacaatct gaataacact gtttatctaa atatcaaaca 2160 ccgttataac attatgaact gaaagacaaa ctgtacttct gacatcctta

ctcagat ttc 2220 ccctaattgt atattcagta tcatttttaaa aaacagattt atattctttt atcagctcag 2280 aaggactagt attcaagaac ccacagaaat ttctctatct tccaagccaa gagcaccaag 2340 aactactccc tttcttcagt tgaatgaaat aagaaatgta aaacatgatg gtaagacact 2400 ttggtgggtttccttcttga agctattatt atcaaattcc ctattcttag gacttgttct 2460 agactaaaag atagttaaga gatatccatc aaatacaatg tatcaaccta aactggatgc 2520 Tggggttctt Tttacaccct ataaaagaca tacctaagac aatcagagaa atacaaatat 2580 ggacttgatt attagataat atagaaggtt tattaatttt cttagatgtg atc atggtat 2640 tgcagtttta aaggagaaca atctcctgtt taagagatac atgctgaaat atttacggag 2700 ttaaaggtca ctggactcca gactggtgat agaacaagac tctgtctcta aaaaataatt 2760 aatttttaa aagaaaatag tttggtaaga tgattcttac attcttaaat aacacgccat 2820 ctaagaaaaa tgctttaaca taaacattac tgaaaaaatg ctacatttgc cacaacttca 2880 taaaatgtca agtgaaatct caagctccaa agatattatt cctattacta aatctgatgt 2940 aataacattt tattgattct ag2962 <210> 2<211> 13<212> DNA<213> Homo sapiens<400> 2tttt ctaaac aag13 <210> 3 <211> 25<212> DNA <213> Homo sapiens <400> 3 gcaggctctg aagctaataa actac25 <210> 4 <211> 25<212> DNA <213> Homo sapiens <400> 4ctagaacaag tcctaagaat aggga25 <210> 5 <211> 25<212> DNA <213> Homo sapiens <400> 5 ggattactag aaatagtgtg cctca 25

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[Translation done.]

L12 ANSWER 1 OF 18 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2003:680767 CAPLUS
DOCUMENT NUMBER: 139:208756
TITLE: Type 2 diabetes risk assessment with angiopoietin-like protein 3 gene polymorphism genotyping
INVENTOR(S): Azumi, Hiroaki; Koishi, Ryuta; Furukawa, Hidehiko
PATENT ASSIGNEE(S): Sankyo Co., Ltd., Japan
SOURCE: Jpn. Kokai Tokkyo Koho, 10 pp.
CODEN: JKXXAF
DOCUMENT TYPE: Patent
LANGUAGE: Japanese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| JP 2003245087 | A2 | 20030902 | JP 2002-338978 | 20021122 |
| PRIORITY APPLN. INFO.: | | | JP 2001-356969 A | 20011122 |
| | | | JP 2001-384102 A | 20011218 |

AB Methods for determining the risk for developing type 2 diabetes mellitus by detecting polymorphism in the angiopoietin-like protein 3 (Angptl3) gene, is disclosed. RFLP, PCR-SSCP, ASO (Allele **Specific** Oligonucleotide) hybridization, direct sequencing, ARMS (Amplification Refracting Mutation System), Denaturing Gradient Gel Electrophoresis, **RNase cleavage**, chemical DNA **cleavage**, DOL (Dye-labeled Oligonucleotide Ligation), Taq Man PCR, invader method, MALDI-TOF/MS (Matrix Assisted Laser Desorption-time of Flight/**Mass Spectrometry**), or TDI (Template-directed Dye-terminator Incorporation), may be used for genotyping. Polymorphism in the intron 2 of Angptl3 gene was genotyped by PCR using primers. A statistically significant difference in allele frequency was found between healthy subjects and type 2 diabetes patients.

L12 ANSWER 4 OF 18 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2000:790667 CAPLUS
DOCUMENT NUMBER: 133:345539
TITLE: Diagnostic sequencing of nucleic acids by a combination of specific cleavage and mass spectrometry
INVENTOR(S): Zabeau, Marc; Stanssens, Patrick
PATENT ASSIGNEE(S): Methexis N.V., Belg.
SOURCE: PCT Int. Appl., 103 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

| PATENT NO. | KIND | DATE | APPLICATION NO. | DATE |
|---|---|---|---|---|
| WO 2000066771 | A2 | 20001109 | WO 2000-EP3904 | 20000430 |
| WO 2000066771 | A3 | 20010208 | | |
| W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM | | | | |
| RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG | | | | |
| CA 2370872 | AA | 20001109 | CA 2000-2370872 | 20000430 |
| EP 1173622 | A2 | 20020123 | EP 2000-940234 | 20000430 |
| EP 1173622 | B1 | 20050615 | | |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, | | | | |